Immunologic Biomarkers in Relation to Exposure Markers of PCBs and Dioxins in Flemish Adolescents (Belgium)

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In this study, we investigated 17- to 18-year-old boys and girls to determine whether changes in humoral or cellular immunity or respiratory complaints were related to blood serum levels of polychlorinated biphenyls (PCBs) and dioxin-like compounds after lifetime exposure in Flanders (Belgium). We obtained blood samples from and administered questionnaires to 200 adolescents recruited from a rural area and two urban suburbs. Physicians recorded medical history and respiratory diseases. We measured immunologic biomarkers such as differential blood cell counts, lymphocyte phenotypes, and serum immunoglobulins. As biomarkers of exposure, we determined the serum concentrations of PCBs (PCB 138, PCB 153, and PCB 180) and dioxin-like compounds [chemical-activated luciferase expression (CALUX) bioassay]. The percentages of eosinophils and natural killer cells in blood were negatively correlated with CALUX toxic equivalents (TEQs) in serum (p = 0.009 and p = 0.05, respectively). Increased serum CALUX TEQs resulted in an increase in serum IgA levels (p = 0.05). Furthermore, levels of specific IgEs (measured by radioallergosorbent tests) of cat dander, house dust mite, and grass pollen were also significantly and negatively associated with the CALUX TEQ, with odds ratios (ORs) equal to 0.63 [95% confidence interval (CI), 0.42–0.96], 0.68 (0.5–0.93), and 0.70 (0.52–0.95), respectively. In addition, reported allergies of the upper airways and past use of antiallergic drugs were negatively associated with CALUX TEQs, with ORs equal to 0.66 (0.47–0.93) and 0.58 (0.39–0.85), respectively. We found a negative association between IgGs and marker PCBs in serum (p = 0.009). This study shows that immunologic measurements and respiratory complaints in adolescents were associated with environmental exposure to polyhalogenated aromatic hydrocarbons (PHAHs). The negative correlation between PHAHs and allergic responses in adolescents suggested that exposure may entail alterations in the immune status. *Key words:* biomonitoring, biomarkers, CALUX, immunotoxicity, polychlorinated biphenyls. *Environ Health Perspect* 110:595–600 (2002). [Online 26 April 2002]
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Polychlorinated aromatic hydrocarbons, including polychlorinated biphenyls (PCBs), polychlorinated dibenzo-*p*-dioxins (PCDDs), and polychlorinated dibenzofurans (PCDFs), are industrial compounds that are contaminants in chemical manufacturing processes or by-products in the combustion of organic materials. They are widely found in the environment and in chemical-waste dump sites. Because of their lipophilic nature, halogenated aromatic compounds bioaccumulate in the food chain. Residues have been detected in foods and in human adipose tissue, milk, and serum fat (*1*).

The toxicity of dioxin-like compounds is mediated through binding to the aryl hydrocarbon receptor (AhR) (*2*). Upon receptor–ligand binding, the complex is translocated to the nucleus and binds to the dioxin-responsive elements of the DNA, which subsequently induces the transcription of genes, for instance, encoding for metabolic enzymes. More recently, interference of polyhalogenated aromatic hydrocarbons (PHAHs) or their metabolites with other hormone receptors has also been observed (*3*).

Polychlorinated aromatic xenobiotics elicit a broad spectrum of biologic and toxic responses. Toxic responses include dermal toxicity, immunotoxicity, carcinogenicity, and adverse effects on reproductive, neurobehavioral, and endocrine functions (*4,5*). Experiments in which laboratory animals and nonhuman primates have been exposed to PCDD/PCDFs and/or PCBs indicate that the immune system is perhaps the most sensitive target for PHAH-induced toxicity. Indeed, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) causes cellular and humoral immune suppression, increased susceptibility to various infectious diseases, thymus atrophy, and depressed antibody and lymphoproliferative responses (*6–11*). Moreover, in wildlife, PCBs/dioxins affect the survival of birds, seals, and beluga whales by diminishing host resistance and increasing incidence and severity of infections (*12,13*).

Accidental or occupational exposure as well as background exposure of the general population to PCBs and dioxins may affect the human immune system (*7,8,14–18*). There is suggestive evidence that dioxin-like compounds influence the immune response by changing the CD4/CD8 ratio, the ratio of other lymphocyte populations, or the antibody production by B cells (*4,7,8,15, 16,18,19*). As in animals, an increased susceptibility to infectious diseases has been noted in adults and children (*16,20,21*).

In this study, we investigated whether lifetime exposure of Flemish adolescents to PCBs and dioxin-like compounds is associated with alterations in the immune system and immunologically mediated health effects.

Methods

Study area. Hoboken and Wilrijk, two adjacent suburbs of the city of Antwerp, Belgium, are located 11–13 km southeast of the chemical and petrochemical industry in Antwerp's seaport. They are also the seat of several small and medium-sized enterprises, a large primary nonferrous smelter (mainly Hoboken), two waste incinerators (Wilrijk), and a crematory (Wilrijk). The two suburbs are traversed by highways with a traffic density > 80,000 vehicles per day. The two waste incinerators near and in Wilrijk have been in operation since 1971 and 1980, respectively. In 1997, when they were shut down, they had annual turnovers of 23,000 and 110,000 tons (*22*). The dioxin levels in topsoil samples obtained in 1997 at a depth of 3–5 cm at 15 sites in a radius of 0.5–3.0 km around the incinerators ranged from 3.9 to 27.2 ng toxic equivalents (TEQ)/kg dry weight (mean, 9.8 ng) (*22*). In contrast, the town of

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Peer and its rural countryside are 15–25 km away from the nearest nonferrous and chemical plants and lie away from major road axes.

Study population. The study population was recruited in 1999, just after the Belgian PCB and dioxin incident (*23*). The target population of the study consisted of 355 adolescents born in 1980–1983 who were in the last 2 years of secondary school and who resided in the suburbs Hoboken and Peer. After informed written consent had been obtained from the parents, 207 adolescents (58% of those invited) agreed to participate. Seven adolescents were excluded from the study because they had recently moved (n = 3) or because they were not immediately available for study because of illness (n = 2) or holidays (n = 2).

Nonresponders had characteristics similar to those of the participants (*24*). The adolescents (100 from Peer, 42 from Wilrijk, and 58 from Hoboken) were examined at their schools between 20 May and 2 December 1999. Examinations started in Peer (May–June), went on in Wilrijk (July–August), resumed in Peer (September–October), and finally took place in Hoboken (November–December).

School physicians recorded medical history (based on the *International Classification of Diseases*) (*25*), in particular, allergic complaints during the past year. Questionnaires were administered to assess lifestyle, dietary habits, smoking and drinking habits, intake of medications, and social class of the parents. Demographic and sociodemographic characteristics of the participants in the three areas are described elsewhere (*24*).

Blood sample collection. We collected blood samples in the morning and separated serum immediately. We divided serum into three parts for individual analysis of immunoglobulins (2 mL), indicator PCB congeners (3 mL), and chemical-activated luciferase expression (CALUX) TEQ (2.5 mL). We immediately froze samples of serum and blood for determination of indicator PCBs; we stored immunoglobulin and CALUX TEQ samples at 4°C; and we transported samples for phenotyping and hematology (at room temperature) to the laboratories within 6 hr for further processing. We performed total and differential blood cell counts on whole blood samples.

Immune phenotyping. We performed two-color flow cytometric immunophenotyping using the lysed whole blood method (Becton Dickinson, San Jose, CA, USA) to determine the following lymphocyte subsets: $CD19^+$ B lymphocytes, $CD3^+$ T lymphocytes, $CD3^+CD4^+$ T-helper lymphocytes, $CD3^+CD8^+$ T-suppressor lymphocytes, $CD45^+$ leukocytes, and $CD16^+CD56^+$ natural killer (NK) cells. We used the following Simultest Kits from Becton-Dickinson: CD45–fluorescein isothiocyanate (FITC), CD3-FITC/CD4-phycoerythrin (PE), CD3-FITC/CD8-PE, CD3-FITC/CD19-PE, CD3-FITC/$CD16^+$CD56-PE, and γ_1-FITC/γ_1-PE control. We stained CD-Chex PLUS from Becton-Dickinson with the same antibodies to serve as an intralab quality control.

We incubated 100 µL aliquots of whole blood with 20 µL antibody for 30 min at room temperature in the dark. After we lysed incubation erythrocytes using Facs lysing solution (Becton-Dickinson), we washed and subsequently fixed them with 1% paraformaldehyde. We performed antibody staining and flow cytometry within 6 hr after blood sampling. We performed lymphocyte gating on forward/sideward scatter dot blots using CD45-FITC–labeled blood cells. Quality control criteria included that the gated population must contain 95% $CD45^+$ cells.

We performed all analyses using a FacsStar Plus cytometer (Becton-Dickinson) equipped with a 488 nm argon air-cooled laser. We used CellQuest software (Becton-Dickinson) for data acquisition and data analyses. Lymphocyte subsets are expressed as percentage of the gated lymphocytes.

Determination of serum immunoglobulins. We used an ELISA method to measure IgA, IgG, IgM, and IgE levels in serum, and we tested hypersensitivity by specific IgE measurements [radioallergosorbent tests (RASTs)]. The antigens tested included house dust mite, cat dander, grass pollen, and birch. We considered the IgE RAST mixtures positive if their value was > 0.70 kU/L.

Indicator PCBs. As described elsewhere (*26,27*), we measured the lower (congeners 28, 52, and 101) and higher (congeners 138, 153, and 180) chlorinated PCBs in serum as biomarkers of exposure to PCBs. Briefly, the samples were vortexed with formic acid for homogenization, followed by two steps of solvent-extraction of PCBs (n-heptane) and purification of extracts by a silica gel column. We performed PCB analyses on a high-resolution gas chromatograph with electron capture detection equipped with two capillary columns of different polarity. We identified the PCBs by means of retention times and carried out quantification using Mirex as internal standard. The detection limit for each congener was 0.015 ng/mL. For internal quality control, we included a blind and a control sample in each series of measurements.

CALUX bioassay. Measurement of the serum dioxin concentration would have required an additional 50 mL of blood. We therefore estimated exposure to dioxin-like compounds via the CALUX bioassay (BioDetection Systems BV, Amsterdam, The Netherlands), an *in vitro* assay that requires only 2.5 mL of serum and is mechanistically based. In this assay, we assessed dioxin-like compounds via *in vitro* activation of the AhR of cultured H4IIE cells (*28–31*).

The method involved n-hexane extraction of 2.5 mL of blood serum and removal of matrix components by passage through a 33% H_2SO_4 silica column. We partly evaporated the extract, quantitatively transferred it to a conical vial for further evaporation, and reconstituted it in dimethyl sulfoxide (Acros Organics, Geel, Belgium) for CALUX measurement using the rat hepatoma H4IIE cell line transfected with an AhR-controlled luciferase reporter gene construct (CALUX assay). We grew cells in 96-well plates in 100 µL of minimal essential medium (Gibco, NV Invitrogen SA, Merelbeke, Belgium) with 10% fetal calf serum (Gibco) at 37°C with 5% CO_2. When the cell layer reached 70–80% confluency, we treated the cells with samples and TCDD standards in quadruplicate and incubated the cells for 24 hr. After removing the medium, we washed the cells with 100 µL phosphate-buffered saline without calcium and magnesium (Gibco) and added 30 µL of cell lysis reagent (Promega, Benelux BV, Leiden, The Netherlands). We then shook the well plates for at least 45 min and stored them at –80°C for at least 1 hr. For determination of luciferase activity, we thawed the cells on ice and added 100 µL of luciferin assay mix (Promega) at room temperature. We measured the light production using a Victor 2 Luminometer (EG&G Wallac, Oosterhout, The Netherlands). We calculated the CALUX-based TEQs by comparing the luciferase activity induced by the sample with a dose–response curve generated from TCDD concentration standards analyzed simultaneously.

Statistical analysis. Database management and statistical analysis were performed with SAS, version 6.12 (SAS Institute, Cary, NC, USA) and Statistica, version 99 (Statsoft, Tulsa, OK, USA). We log-transformed data that were not normally distributed and described continuous data by the arithmetic mean ± 95% confidence interval (CI) or the geometric mean with 95% CI. We used dichotomous classifications to code for the presence of allergic diseases and positive allergic tests. We used the Student's t-test and Fisher's exact test to compare means and proportions, respectively, between girls and boys.

We identified confounding variables by stepwise multiple regression or logistic regression. The p-value for variables to enter and to stay in the model was set at 0.05. We checked the following covariables: sex, smoking habits, alcohol consumption, history of infectious or allergic diseases, familial

history of hay fever or asthma, maternal smoking habits during pregnancy, having been breast-fed, body mass index, social class of the parents, use of oral contraceptives, and mean atmospheric ozone concentrations and mean daily temperatures during the week before blood sampling (both obtained from the Royal Meteorological Institute, Brussels, Belgium).

We calculated dose–effect relations in individual subjects between the biomarkers of immunologic effects and those reflecting exposure to PHAHs, using multiple linear regression for continuous outcomes or logistic regression for categorical variables.

Results

Characteristics of the participants. The 200 adolescents (mean age ± SD, 17.4 ± 0.8 years) included 120 girls (60%). Mean age (17.3 vs. 17.4 years), mean body mass index (21.2 kg/m^2 vs. 21.1 kg/m^2), proportions of current smokers (25%), social class of parents (23% workers, 64% middle class, 12% educated professionals), and breast-fed subjects (56%) were similar in girls and boys. Compared with girls, more boys consumed alcohol (29% vs. 65%). Among the girls, 41% were on oral contraceptives.

The red blood cell count and the total and differential white blood cell counts are shown in Table 1. The hematologic measurements were within the normal ranges.

The lower chlorinated PCB congeners 28, 53, and 101 in the adolescents were all below their respective limits of detection (0.16, 0.21, and 0.18 nmol/L). PCB congener 153 represented the major fraction (46%) of the combined marker PCBs, and congeners 138 and 180 each accounted for 27% of the total. For all further analyses, we combined congeners 138, 153, and 180.

The mean serum concentrations of the marker PCBs were 0.99 and 1.67 nmol/L in girls and boys, respectively, whereas CALUX TEQ was similar in both sexes, 0.15 and 0.16 pg/mL serum, respectively (Table 1).

The percentages of adolescents with positive RAST tests or positive personal or familial histories of allergic or bronchial disorders appear in Table 2.

Dose–effect relations. We computed dose–effect (Table 3) and dose–response (Table 4) relationships between the biomarkers of exposure and various immunologic measurements or the odds of showing a positive test or history of allergic or bronchial disorders. We tested a large number of potential confounding variables. The following variables did not reach statistical significance, and were not included in any of the regression models: alcohol consumption, smoking of mother during pregnancy, having been breast-fed, body mass index, social class of parents, use of oral contraceptives, mean atmospheric ozone concentrations, and mean daily temperatures during the week before blood sampling. The variables for which our analyses were adjusted are listed in Tables 3 and 4.

The eosinophil count was negatively and independently correlated with the serum concentrations of dioxin-like compounds (p = 0.009; Table 3). Monocytes tended to decrease with increasing serum TEQ values (p = 0.055; Table 3). We observed a negative association, although at borderline significance, between the number of NK cells ($CD16^+CD56^+$) and the serum concentration of dioxin-like compounds (p = 0.05; Table 3). We found no significant associations between the other lymphocyte phenotypes and either serum TEQ values or the combined serum concentrations of PCB congeners 138, 153, and 180 (Table 3). We obtained similar results when we expressed lymphocyte subpopulations as absolute numbers (cells per milliliter).

The dioxin-like activity in the serum was negatively correlated with serum IgE levels (p = 0.02) but positively correlated with IgA concentrations (p = 0.05; Table 3). We found a negative correlation between IgG levels and the concentration of the combined marker PCBs (p = 0.009; Table 3).

After adjustment for sex and familial history of hay fever, serum TEQ values were negatively associated with the odds of having a positive RAST for house dust mites [odds ration (OR) = 0.68; p = 0.01], cat dander (OR = 0.63; p = 0.03), and grass pollen (OR = 0.70; p = 0.02; Table 4). A history of upper airway allergy was negatively associated with serum TEQ values (OR = 0.66; p = 0.02; Table 4).

Respiratory complaints (Table 4) were not confounded by meteorological conditions, such as mean daily temperature and ozone concentration. We found a negative association between the odds of bronchial wheezing and serum CALUX TEQ (OR = 0.25; p = 0.03). After adjustment for familial history of hay fever and/or asthma, the significance disappeared (OR = 0.72; p = 0.07). Before and after similar adjustments, a positive answer to the question "ever received medication against asthma" was negatively associated with serum CALUX TEQ (OR = 0.58; p = 0.005). "Ever asthma" was positively associated with the serum concentration

Table 1. Hematologic, immunologic, and exposure measurements in 200 adolescents.

Measurement (unit)	Girls (n = 120) Mean	Girls (n = 120) 95% CI	Boys (n = 80) Mean	Boys (n = 80) 95% CI	p-Value[a]
Red blood cells (E12/L)	4.55	4.50–4.61	5.07	4.95–5.19	< 0.0001
White blood cells (E9/L)	6.32	6.05–6.59	5.82	5.61–6.18	0.03
Lymphocytes (%)	31.56	30.23–32.89	33.53	31.77–35.30	0.07
Monocytes (%)	6.87	6.56–7.19	7.72	7.04–8.05	0.0004
Trombocytes (E9/L)	258.26	244.69–271.83	223.70	207.66–239.74	0.0014
Eosinophils[b] (%)	2.10	1.88–2.34	3.03	2.59–3.53	0.0001
CD3 (%)	66.53	65.21–67.84	60.11	58.40–61.83	< 0.0001
CD4 (%)	38.28	37.10–39.47	33.64	32.26–35.03	< 0.0001
CD8 (%)	21.32	20.35–22.28	20.56	19.38–21.73	0.32
CD19 (%)	13.15	12.35–14.00	14.57	13.63–15.50	0.03
CD45 (%)	96.87	96.08–97.66	96.9	96.57–97.23	0.96
CD4/CD8	1.87	1.76–1.97	1.74	1.62–1.87	0.15
$CD16^+CD56$[b] (%)	14.42	13.27–15.67	17.70	16.26–19.27	0.002
IgA[b] (mg/dL)	1.35	1.26–1.45	1.49	1.35–1.65	0.11
IgE[b] (kIU/L)	17.66	12.85–24.32	41.3	25.82–66.07	0.002
IgG[b] (mg/dL)	10.09	9.71–10.50	9.51	9.06–10.0	0.07
IgM[b] (mg/dL)	1.27	1.17–1.37	0.90	0.82–0.99	< 0.0001
CALUX TEQ[b] (pg TEQ/mL)	0.15	0.13–0.17	0.16	0.14–0.19	0.45
CALUX TEQ[b] (pg TEQ/g fat)	28.59	24.93–32.80	34.89	28.66–42.46	0.09
Sum marker PCBs[b,c] (nmol/L)	0.99	0.90–1.09	1.67	1.51–1.83	< 0.0001
Sum marker PCBs[b,c] (pmol/g fat)	189.67	172.19–208.45	359.75	326.59–397.19	< 0.0001

Values are arithmetic means with 95% CI except where indicated.
[a]Significance of the difference between girls and boys (Student's t-test). [b]Geometric means (logarithmically transformed distribution) with 95% CI. [c]Sum of congeners 138, 153, and 180.

of marker PCBs (OR = 2.12; p = 0.05) even after correction. The odds of suffering from hay fever increased with higher serum PCB concentrations (OR = 1.63; p = 0.04). However, after correcting for sex, significance disappeared, because in our study, hay fever was more frequently reported by boys (31%) than by girls (17%).

When we expressed the serum concentrations of PCBs or dioxin-like compounds per gram of fat rather than per volumetric unit, dose–effect and dose–response relationships were similar.

Discussion

In this study we report on the immune status of 200 Flemish adolescents in relation to their exposure to PCBs and dioxins. The serum concentration of dioxin-like compounds was negatively and independently correlated with a history of upper airway allergy and "ever received medication against asthma," with eosinophil counts, with serum concentrations of IgEs, and with the odds of having a positive RAST for house dust mites, cat dander, and grass pollen. The serum concentration of dioxin-like compounds was negatively associated with the proportions of NK cells and monocytes but positively associated with serum IgA levels. The changes in the immune system may reflect a decreased susceptibility to allergic reactions, as suggested by Weisglas-Kuperus (*18*).

In the present study we measured the serum concentrations of dioxin-like compounds by the mechanistically based CALUX bioassay, which measures all compounds in the serum that act via binding to the AhR. This direct toxicity measure of dioxin-like activity may be a more relevant exposure estimate than chemical analyses of individual dioxin/furan congeners that are added after multiplication with their respective toxic equivalent factors (TEF values). Furthermore, we determined PCB congeners 138, 153, and 180, which account for only 40–60% of the total PCB burden (*32*). Only PCB 118 has a weak dioxin activity, with a TEF value of 0.0001 (*33*). We less frequently found significant associations between immune effects and the combined levels of marker PCBs. The PCB congeners that we measured in this study may not be those with the greatest toxicity to the immune system.

Multiple cellular targets within the immune-hematopoietic system can be altered by dioxin-like compounds (*34,35*). In experimental systems (animal and *in vitro* experiments), PCB/dioxin exposure leads to suppression of humoral and cell-mediated immunity and has direct effects on hematopoietic stem cells and B-cell or T-cell differentiation (*8,11,36,37*). However, the underlying mechanisms for these effects have not been fully elucidated. Epidemiologic studies have shown modulation of white blood cell counts, distribution of lymphocyte subsets, and the amount of immunoglobulins in serum by PCB/PCDD exposure (*7,8,10*). However, published data on the effects of dioxin-like compounds on these measurements are often incomplete. Studies often lack individual quantification of the body

Table 2. Percentage of adolescents with positive RASTs or positive personal or familial history of allergic or bronchial disorders.

Positive test or history	Girls (n = 120)	Boys (n = 80)	p-Value[a]
RAST			
House dust mite	18.3	31.3	0.04
Cat dander	5.8	13.7	0.08
Grass pollen	19.2	37.5	0.005
Birch	4.2	20.0	0.0006
Overall[b]	27.5	46.3	0.01
History of allergic disease			
Upper airways	11.7	20.0	0.11
Lower airways	3.3	10.0	0.07
Eyes	0	1.3	0.40
Skin	7.5	10.0	0.61
Overall	20.0	32.5	0.07
History of infectious disease			
Bacterial infections	8.3	1.3	0.05
Viral infections	57.5	58.8	0.88
Overall infections	60.8	60.0	1.00
History of respiratory symptoms			
Bronchial wheezing	19.2	13.8	0.34
Hay fever	16.7	31.3	0.02
Ever asthma	6.7	11.3	0.31
Asthma attack last year	3.3	2.5	0.66
Familial history			
Hay fever	45.0	27.5	0.01
Asthma	15.8	12.5	0.54

[a]Significance of the difference between girls and boys (Fisher's exact test). [b]Positive to any of the four RASTs.

Table 3. Dose–effect relationships between various immunologic measurements and the biomarkers of exposure to polychlorinated hydrocarbons.

	Dioxin-like compounds in serum (pg CALUX TEQ/mL)			Combined marker PCBs (nmol/L)[a]		
Biomarker of effect (unit)	Partial correlation coefficient r	Estimate (SE)	p-Value	Partial correlation coefficient r	Estimate (SE)	p-Value
Red blood cells (E12/L)	–0.11	–0.408 (0.24)	0.09	–0.03	–0.007 (0.02)	0.66
White blood cells (E9/L)	0.10	1.177 (0.87)	0.18	0.06	0.044 (0.06)	0.48
Lymphocytes (%)	–0.07	–4.368 (4.28)	0.31	–0.11	–0.399 (0.30)	0.18
Monocytes (%)	–0.13	–1.699 (0.88)	0.055	–0.10	–0.082 (0.06)	0.19
Thrombocytes (E9/L)	0.015	8.80 (41.29)	0.83	–0.03	–1.04 (2.90)	0.72
Eosinophils (%)	–0.18	–0.408 (0.15)	0.009	0.005	0.0008 (0.01)	0.95
CD3 (%)	0.09	5.87 (4.18)	0.16	0.05	0.19 (0.28)	0.49
CD4 (%)	0.05	2.46 (3.60)	0.49	0.10	0.35 (0.25)	0.16
CD8 (%)	0.03	1.31 (3.0)	0.66	–0.05	–0.13 (0.21)	0.54
CD19 (%)	0.02	0.81 (2.45)	0.74	0.04	0.08 (0.17)	0.64
CD45 (%)	0.03	0.93 (2.0)	0.64	–0.05	–0.09 (0.14)	0.53
$CD16^{+}CD56$ (%)	–0.13	–0.20 (0.1)	0.05	–0.08	–0.29 (0.26)	0.27
CD4/CD8	0.01	0.04 (0.33)	0.91	0.11	0.03 (0.02)	0.16
IgA (mg/dL)	0.14	0.20 (0.1)	0.05	0.06	0.005 (0.007)	0.47
IgE (kIU/L)	–0.16	–1.08 (0.46)	0.02	–0.04	–0.015 (0.03)	0.65
IgG (mg/dL)	0.04	0.03 (0.05)	0.56	–0.20	–0.009 (0.004)	0.009
IgM (mg/dL)	–0.01	–0.02 (0.1)	0.88	0.002	0.0002 (0.007)	0.97

We adjusted the partial correlation coefficients for sex and current smoking.
[a]Sum of congeners 138, 153, and 180.

burden or report only between-group differences instead of dose–effect or dose–response relationships. In the adolescents in this study, we found significant associations between biomarkers of immunologic status and biomarkers of internal PHAH exposure.

Changes in cell surface markers on T cells represent an apparently sensitive biomarker response to the effects of dioxin-like compounds in rodents and primates. Dioxin-like compounds can affect the primary immune response by changing the CD4/CD8 ratio or the ratio of other lymphocyte subpopulations (*11,15,19,38–41*). In the present study we found no associations between T-helper or T-suppressor cells and the serum PCB/CALUX TEQ levels. In our adolescents, the numbers of NK cells ($CD16^+CD56^+$ cells), eosinophils, and monocytes showed negative correlations with the concentration of dioxin-like compounds in the serum. These findings are in agreement with a study in Dutch infants in which exposure was associated with lower monocyte counts (*21*) and with a Swedish study that indicated that consumers of persistent organochlorine compound-contaminated fish had lower proportions and numbers of NK cells (*42*).

Reduced numbers of monocytes and NK cells ($CD16^+CD56^+$ cells) may be an indication of depressed cellular immunity (*34*). However, the present associations were of borderline significance and should be carefully interpreted. The body burden of dioxin-like substances in our young study group could have been too low to induce considerable alterations in the subpopulation of white blood cells. Alternatively, the immunosuppressive effect of dioxin-like compounds might be mediated by a decreased functionality of individual cells rather than by a reduction in absolute cell numbers in the peripheral blood.

In humans, dioxin-like compounds can act on B cells, resulting in an impairment of antibody production (*40,43*). In our study we measured the serum concentrations of immunoglobulins (IgA, IgM, IgG, IgE) as biomarkers of humoral immunity. We observed a positive correlation between serum IgA and serum TEQ/mL but a negative correlation between serum IgE and serum TEQ/mL. Both correlations were weak and only borderline significant. Furthermore, in experimental animals such as monkeys, PCBs affect the primary antibody response, as evidenced by the depressed antibody response to sheep red blood cells (*44,45*). However, in the present study none of the serum immunoglobulins correlated with the combined concentration of marker PCBs in serum.

Positive associations between serum IgA levels and TCDD exposure have been found in the residents of Missouri as well as in Vietnam veterans (*40,46*). The latter have been exposed to TCDD through use of the pesticide Agent Orange.

Because serum immunoglobulin levels do not necessarily reflect the specific immune responses to common respiratory allergens, we also performed RASTs. We noticed a negative association between the odds of having a positive response to house dust mites, cat dander, or grass pollen and the serum concentration of dioxin-like compounds. To the best of our knowledge, data on specific IgE levels in relation to PCB/TCDD exposure levels have not yet been reported. Reduced antigen-specific IgG antibody responses after mumps and rubella vaccination have been reported in perinatally exposed Dutch children (*18*).

Immune deficiency generally manifests as an increased susceptibility to infections. Increased infection rates (e.g., severe cases of the common cold) are difficult to ascertain in epidemiologic surveys (*34,35*). Wildlife populations show increased susceptibility to bacterial and viral infections after PCB/TCDD exposure (*12,13*). Mononuclear phagocytic cells of PCB-exposed experimental animals show reduced phagocytic activity (*39*). In animals, therefore, PCB exposure may be associated with a decreased clearance of pathogenic bacteria by the spleen and liver, a diminished resistance to viruses, and an increased sensitivity to bacterial endotoxins (*7*). One-year-old Inuits who were breast-fed with milk contaminated with PCBs (621 μg/kg) showed a 20-fold higher incidence of infectious diseases, such as measles, meningitis, and otitis media compared with age-matched controls (*20*). Yucheng children born between July 1978 and June 1987 to women accidentally exposed to PCBs/PCDFs through the consumption of contaminated rice bran oil showed a higher rate of bronchitis, compared with controls, in the first 6 months after birth and higher frequencies of respiratory tract infections and otitis media attacks in a 6-year follow-up (*47*). Higher prevalences of recurrent middle-ear infections and of chicken pox were positively associated with current PCB body burden in Dutch preschool children (*18*). A higher dioxin TEQ was also associated with a higher prevalence of coughing, chest congestion, and phlegm (*18*). The same Dutch study (*18*) showed a negative association between prenatal PCB exposure and shortness of breath with wheezing, whereas the current PCB burden was associated with a lower prevalence of allergic reactions. Suppression of the allergic immune response after TCDD exposure was recently observed in a rat model (*48*). In this study, history of allergic and respiratory complaints were also negatively associated with the serum concentration of dioxin-like activity.

In conclusion, we found in 17- to 18-year-old adolescents that biomarkers of internal exposure to PHAHs, in particular, dioxin-like compounds, were related to biomarkers of immune status. The effects of exposure to dioxin-like compounds in adolescents were associated with a lower prevalence of allergic diseases.

Table 4. Dose–response relationship between the odds of a positive RAST or history of allergic or bronchial disease and the biomarkers of exposure to polychlorinated hydrocarbons.

	Dioxin-like compounds in serum (pg CALUX TEQ/mL)		Combined marker PCBs (nmol/L)[a]	
Biomarker of effect	OR (95% CI)	*p*-Value	OR (95% CI)	*p*-Value
Positive RAST[b]				
House dust mite	0.68 (0.50–0.93)	0.01	0.77 (0.45–1.31)	0.33
Cat dander	0.63 (0.42–0.96)	0.03	0.99 (0.44–2.22)	0.98
Grass pollen	0.70 (0.52–0.95)	0.02	1.17 (0.68–1.98)	0.57
Birch	0.77 (0.51–1.14)	0.19	0.91 (0.36–1.32)	0.53
Overall	0.77 (0.58–1.01)	0.06	1.03 (0.64–1.66)	0.92
History of allergic disease[b]				
Upper airways	0.66 (0.47–0.93)	0.02	1.18 (0.62–2.25)	0.61
Lower airways	0.87 (0.52–1.47)	0.61	0.94 (0.34–2.55)	0.90
Skin	0.97 (0.62–1.51)	0.88	1.49 (0.66–3.33)	0.34
Overall	0.76 (0.57–1.03)	0.08	1.23 (0.71–2.11)	0.46
History of infectious disease				
Bacterial infections	1.86 (0.54–6.42)	0.32	0.97 (0.88–1.07)	0.54
Viral infections	1.38 (0.69–2.76)	0.26	0.97 (0.93–1.00)	0.09
Overall infections	1.30 (0.65–2.62)	0.46	0.96 (0.93–1.01)	0.15
History of respiratory symptoms[c]				
Bronchial wheezing	0.72 (0.51–1.03)	0.07	1.18 (0.69–2.04)	0.55
Hay fever	0.97 (0.71–1.33)	0.86	1.63 (1.02–2.61)	0.04
Ever asthma	0.75 (0.49–1.15)	0.19	2.12 (1.01–4.46)	0.05
Ever medication against asthma	0.58 (0.39–0.85)	0.005	1.02 (0.56–1.87)	0.95
Breathless while wheezing	0.90 (0.46–1.76)	0.75	1.11 (0.46–2.67)	0.81
Wheezing during exertion	0.69 (0.35–1.37)	0.27	1.16 (0.52–2.62)	0.70

[a]Sum of congeners 138, 153, and 180. [b]Adjusted for sex and family history of hay fever. [c]Adjusted for family history of hay fever and/or asthma.

References and Notes

1. Safe S. Polychlorinated biphenyls (PCBs), Dibenzo-*p*-dioxins (PCDDs), dibenzofurans (PCDFs), and related compounds: environmental and mechanistic considerations which support the development of toxic equivalency factors (TEFs). Crit Rev Toxicol 21:51–88 (1990).
2. Rowlands JC, Gustafsson JA. Aryl hydrocarbon receptor-mediated signal transduction. Crit Rev Toxicol 27(2):109–134 (1997).
3. McClellan RO. Dioxin-like and non-dioxin-like toxic effects of polychlorinated biphenyls (PCBs): implications for risk assessment. Crit Rev Toxicol 28(6):511–570 (1998).
4. Weisglas-Kuperus N. Neurodevelopmental, immunological and endocrinological indices of perinatal human exposure to PCBs and dioxins. Chemosphere 37(9–12):1845–1853 (1998).
5. Lindström G, Hooper K, Petreas M, Stephens R, Gilman A. Workshop on perinatal exposure to dioxin-like compounds. V. Summary. Environ Health Perspect 103(suppl 2):135–142 (1995).
6. Holsapple MP, Snyder NK, Wood SC, Morris DL. A review of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin-induced changes in immunocompetence: 1991 update. Toxicology 69:219–255 (1991).
7. Tryphonas H. Immunotoxicity of PCBs (Aroclors) in relation to Great Lakes. Environ Health Perspect 103(suppl 9):35–46 (1995).
8. Kerkvliet NI. Immunological effects of chlorinated dibenzo-*p*-dioxins. Environ Health Perspect 103(suppl 9):47–53 (1995).
9. De Waal EJ, Schuurman HJ, Van Looveren H, Vos J. Differential effects of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin, bis(tri-*N*-butyltin)oxide and cyclosporine on thymus histophysiology. Crit Rev Toxicol 27:381–430 (1997).
10. Golden RJ, Noller KL, Titus-Ernstoff L, Kaufman RH, Mittendorf R, Stillman R, Reese EA. Environmental endocrine modulators and human health: an assessment of the biological evidence. Crit Rev Toxicol 28:109–227 (1998).
11. Neubert R, Stahlmann R, Korte M, van Loveren H, Vos JG, Webb JR, Golor G, Helge H, Neubert N. Effects of small doses of dioxins on the immune system of marmosets and rats. Ann NY Acad Sci 686:662–686 (1993).
12. Ross P, De Swaert R, Addison R, Van Looveren H, Vos J, Osterhaus A. Contaminant-induced immunotoxicity in harbour seals: wildlife at risk? Toxicology 112:157–169 (1996).
13. Van Looveren H, Ross PS, Osterhaus AD, Vos JG. Contaminant-induced immunosuppression and mass mortalities among harbor seals. Toxicol Lett 15 (112-113):319–324 (2000).
14. Tonn T, Esser C, Schneider EM, Steinmann-Steiner-Haldenstätt W, Gleichman E. Persistence of decreased T-helper function in industrial workers 20 years after exposure to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin. Environ Health Perspect 104:422–426 (1996).
15. Ernst M, Flesch-Janys D, Morgenstern I, Manz A. Immune cell functions in industrial workers after exposure to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin: dissociation of antigen-specific T-cell responses in cultures of diluted whole blood and of isolated peripheral blood mononuclear cells. Environ Health Perspect 106(suppl 2):701–705 (1998).
16. Jung D, Berg PA, Edler L, Ehrenthal W, Fenner D, Flesch-Janys D, Huber C, Klein R, Koitka C, Lucier G, et al. Immunologic findings in workers formerly exposed to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin and its congeners. Environ Health Perspect 106(suppl 2):689–695 (1998).
17. Birnbaum LS. Workshop on perinatal exposure to dioxin-like compounds. V. Immunologic effects. Environ Health Perspect 103(suppl 2):157–160 (1995).
18. Weisglas-Kuperus N, Patandin S, Berbers GAM, Sas TCJ, Mulder PGH, Sauer PJJ, Hooijkaas H. Immunologic effects of background exposure to polychlorinated biphenyls and dioxins in Dutch preschool children. Environ Health Perspect 108:1203–1207 (2000).
19. Nagayama J, Tsuji H, Iida T, Hirakawa H, Matsueda T, Okamura K, Hasegawa M, Sato K, Ma HY, Yanagawa T, et al. Postnatal exposure to chlorinated dioxins and related chemicals on lymphocyte subsets in Japanese breast-fed infants. Chemosphere 37(9–12):1781–1787 (1998).
20. Dewailly E, Ayotte P, Bruneau S, Gingras S, Belles-Isles M, Roy R. Susceptibility to infections and immune status in Inuit infants exposed to organochlorines. Environ Health Perspect 108:205–211 (2000).
21. Weisglas-Kuperus N, Sas T, Koopman-Esseboom C, van der Zwan C, de Ridder M, Beishuizen A, Hooijkaas H, Sauer P. Immunological effects of background prenatal and postnatal exposure to dioxins and polychlorinated biphenyls in Dutch infants. Pediatr Res 38:404–410 (1995).
22. Schoeters G, Cornelis C, De Fré R, Hooghe R, Collier M, Maes A, Mensinck C, Nouwen J, Patyn J, Verschaeve L, et al. Studie van de gezondheidsaspecten en gezondheidsrisico's ten gevolge van de milieuverontreiniging in de Neerlandwijk te Wilrijk. Studie uitgevoerd in opdracht van het Ministerie van de Vlaamse Gemeenschap. Report number 1998/TOX/R/097. Mol, Belgium:Departement Gezondheidsbeleid, 1998.
23. van Larebeke N, Hens L, Schepens P, Covaci A, Baeyens J, Everaert K, Bernheim JL, Vlietinck R, De Poorter G. The Belgian PCB and dioxin incident of January–June 1999: exposure data and potential impact on health. Environ Health Perspect 109:257–265 (2001).
24. Staessen JA, Nawrot T, Den Hond E, Thijs L, Fagard R, Hoppenbouwers K, Koppen G, Nelen V, Schoeters G, Vanderschueren D, et al. Renal function, cytogenetic measurements, and sexual development in adolescents in relation to environmental pollutants: a feasibility study of biomarkers. Lancet 357:1660–1669 (2001).
25. WHO. Manual of the International Statistical Classification of diseases, injuries, and causes of death, Vol 1. Geneva:World Health Organization, 1967.
26. Hansen LG. Stepping backward to improve assessment of PCB congener toxicities. Environ Health Perspect 106(suppl 1):171–189 (1998).
27. Fastabend A. Determination of PCBs and organochlorpesticides at environmental concentrations by means of GC-ECD using optimized procedures for sample preparation [PhD Thesis]. Chlausthal, Germany:Universitat Chlausthal, 2000.
28. Murk AJ, Legler J, Denison MS, Giey JP, Van de Guchte C, Brouwer A. Chemical-activated luciferase expression (CALUX): a novel in vitro bioassay for Ah receptor active compounds in sediments and pore water. Fundam Appl Toxicol 33:149–160 (1996).
29. Aerts JMMJG, Cenijn PH, Blankvoort BMG, Murk AJ, Bovee TFH, Traag WA. Application of the chemical activated luciferase expression (CALUX) bioassay for quantification of dioxin-like compounds in small samples of human milk and blood plasma. Organohalogen Comp 27:285–290 (1996).
30. Murk AJ, Leonards PEG, van Hattum B, Luit R, van der Weiden MEJ, Smit M. Application of biomarkers for exposure and effect of polyhalogenated aromatic hydrocarbons in naturally exposed European otters (*Lutra lutra*). Environ Toxicol Pharmacol 6:91–102 (1998).
31. Koppen G, Covaci A, Van Cleuvenbergen R, Schepens P, Winneke G, Nelen V, Schoeters G. Comparison of CALUX-TEQ values with PCB and PCDD/F measurements in human serum of the Flanders Environmental and Health Study (FLEHS). Toxicol Let 123:59–67 (2001).
32. Brouwer A, Ahlborg UG, Vandenberg M, Birnbaum LS, Boersma ER, Bosveld B, Denison MS, Gray LE, Hagmar L, Holene E, et al. Functional aspects of development toxicity of polyhalogenated aromatic-hydrocarbons in experimental animals and human infants. Eur J Pharmacol Environ Toxicol Pharmacol 293:1–40 (1995).
33. Van den Berg M, Birnbaum LS, Bosveld ATC, Brunstrom B, Cook P, Feeley M, Giesy JP, Hanberg A, Hasegawa R, Kennedy SW, et al. Toxic equivalency factors (TEFs) for PCBs, PCDDs, and PCDFs for humans and wildlife. Environ Health Perspect 106:775–792 (1998).
34. WHO. Principles and Methods for Assessing Direct Immunotoxicity Associated with Exposure to Chemicals. Environmental Health Criteria 180. Geneva:World Health Organization, 1996.
35. Straight JM, Kipen HM, Vogt RF Jr, Amler RW. Immune function test batteries for use in environmental health field studies. Publication no. PB94–204328. Springfield, VA:National Technical Information Service, 1994.
36. Neubert R, Jacob-Müller U, Helge H, Stahlmann R, Neubert D. Polyhalogenated dibenzo-*p*-dioxins and dibenzofurans and the immune system 2. In vitro effects of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) on lymphocytes of venous blood from man and a non-human primate (*Callithrix jacchus*). Arch Toxicol 65:213–219 (1991).
37. Sulentic CEW, Holsapple MP, Kaminsli NE. Aryl hydrocarbon receptor-dependent suppression by 2,3,7,8-tetrachlorodibenzo-*p*-dioxin of IgM secretion in activated B cells. Mol Pharmacol 53:623–629 (1998).
38. Kimbrough RD. Polychlorinated biphenyls (PCBs) and human health: an update. Crit Rev Toxicol 25:133–163 (1995).
39. Tryphonas H, Luster MI, Schiffman G, Dawson LL, Hodgen M, Germolec D, Hayward S, Bryce F, Loo JCK, Mandy F, et al. Effect of chronic exposure of PCB (Arochlor 1254) on specific and nonspecific immune parameters in the rhesus (*Macaca mulatta*) monkey. Fundam Appl Toxicol 16:773–786(1991).
40. Webb KB, Evans RG, Knutsen AP, Roodman ST, Roberts DW, Schramm WF, Gibson BB, Andrews JS, Needham LL, Patterson DG. Medical evaluation of subjects with known body levels of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin. J Toxicol Environ Health 28:183–193 (1989).
41. Chang KJ, Hsieh KH, Lee TP, Tang SY, Tung TC. Immunological evaluation of patients with polychlorinated biphenyl poisoning: determination of lymphocyte subpopulations. Toxicol Appl Pharmacol 61:58–63 (1981).
42. Svensson BG, Hallberg T, Nilsson A, Schutz A, Hagmar L. Parameters of immunological competence in subjects with high consumption of fish contaminated with persistent organochlorine compounds. Int Arch Occup Environ Health 65:351–358 (1994).
43. Lu Y-C, Wu Y-C. Clinical findings and immunological abnormalities in Yu-cheng patients. Environ Health Perspect 59:17–29 (1985).
44. Davis D, Safe S. Dose-response immunotoxicities of commercial polychlorinated biphenyls (PCBs) and their interaction with 2,3,7,8-tetrachloredibenzo-*p*-dioxin. Toxicol Lett 48:35–43 (1989).
45. Arnold DL, Mes J, Bryce F, Karpinski K, Bickis MG, Zawidska Z, Stapley R. A pilot study on the effects of Aroclor 1254 ingestion by rhesus and cynomolgus monkeys as a model for human ingestion of PCBs. Food Chem Toxicol 28:847–857 (1990).
46. Roegner RH, Grubbs WD, Lustik MB, Brockman AS, Henderson SC, Williams DE, Wolfe WH, Michalek JR, Miner JC. Air Force Health Study: An Epidemiologic Investigation of Health Effects in Air Force Personnel Following Exposure to Herbicides. NTIS #AD A-237–516 through AD A-237–524. Springfield, VA:National Technical Information Service, 1991.
47. Yu ML, Hsu CC, Chan WC, Guo YL. The immunologic evaluation of the Yucheng children. Chemosphere 37:1855–1865 (1998).
48. Luebke RW, Copeland CB, Daniels M, Lambert AL, Gilmour MI. Suppression of allergic immune responses to house dust mite (HDM) in rats exposed to 2,3,7,8-TCDD. Toxicol Sci 62:71–79 (2001).